Understanding the key questions of radiation combined injuries (RCI)

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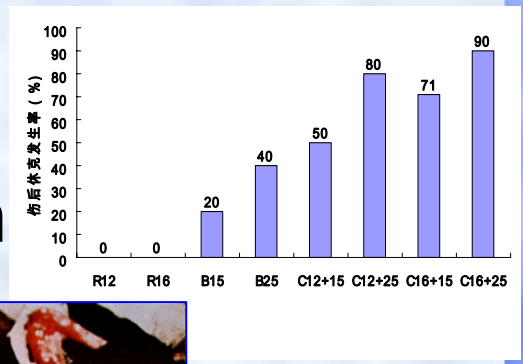
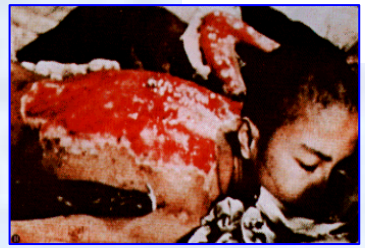
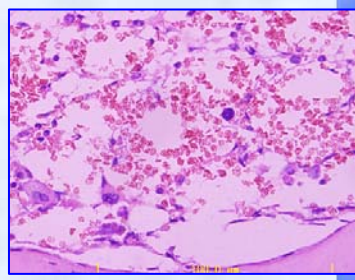
Key questions of RCI



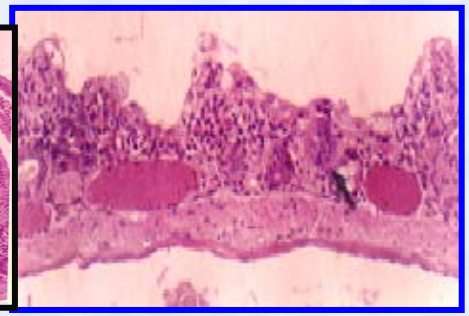
- Severer, and earlier shock -- death causing



- Refractory wound healing
- Hematopoiesis suppression & reconstruction -- affect all of the clinic duration



- Extensive and severe GI damage (mechanical & immune barrier)



Our goals

Radiation Combined Injury

- **Hematopoiesis**
- **Intestine**
- **Wound**
- **Shock**

Combined Effect & its mechanism

Rescue & Treatment

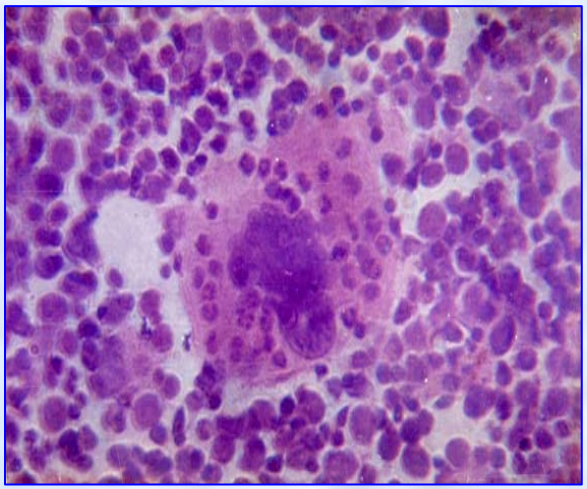
1. Hematopoiesis suppression & reconstruction

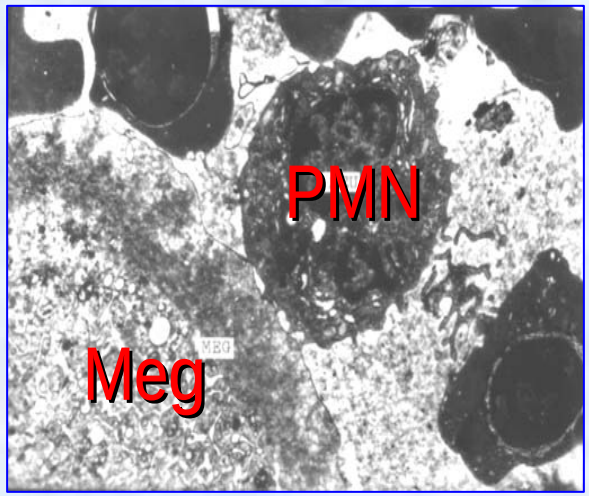
- Elucidated the mechanism of magekaryocyte (Meg) damage after RCI
- A new type of fusion protein stimulating thrombopoiesis was developed and tested in animals.

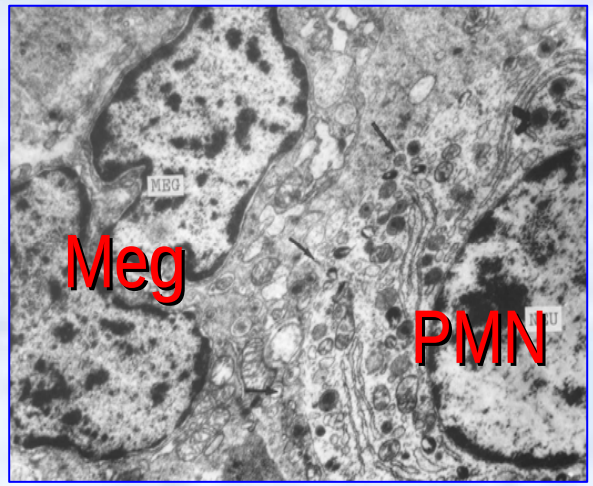
Unsolved questions

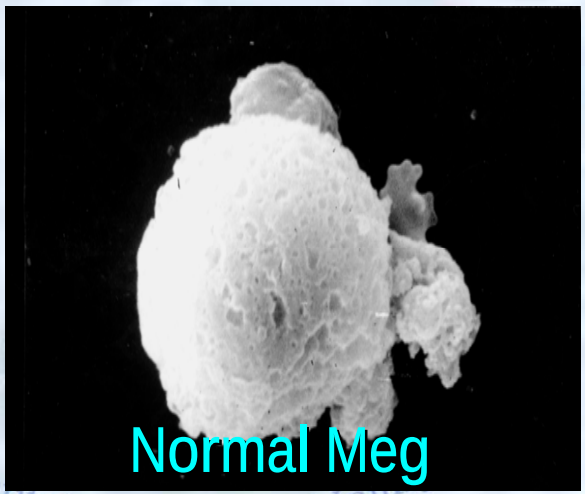
- **Comprehensive studies on granulocytes and erythrocytes, but rarely on megakaryocyte/platelet**
- **Limited regimen available for accelerating thrombogenesis comparing to that for other lineages**

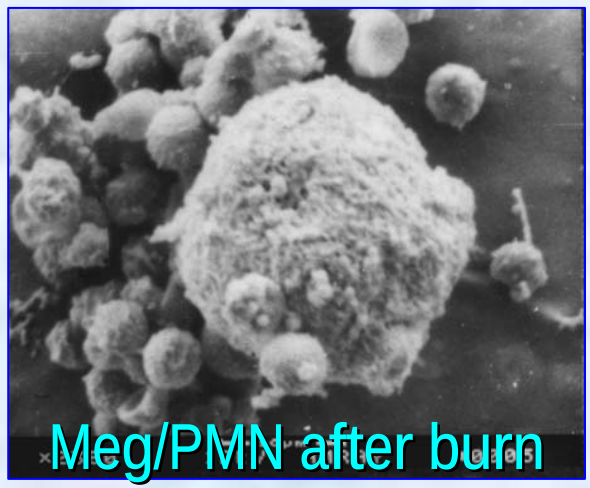
Megakaryocytophagia





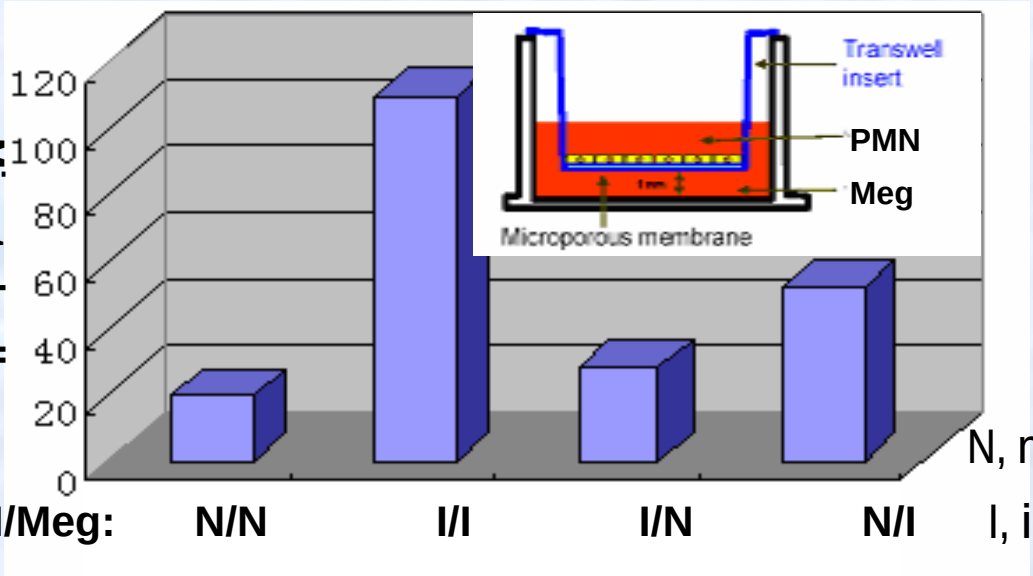






Interaction between Meg and PMN

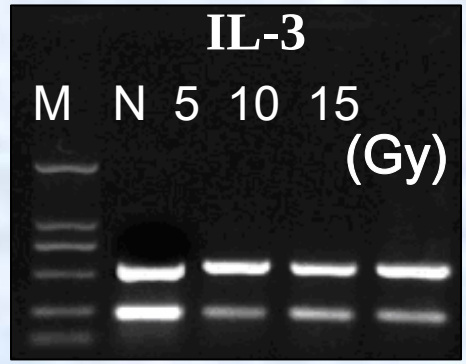
- PMNs more active in engulfment after injury
- Injured Meg still chemoattracted PMNs
- Anti-IL8,Verapamil inhibited PMNs migration

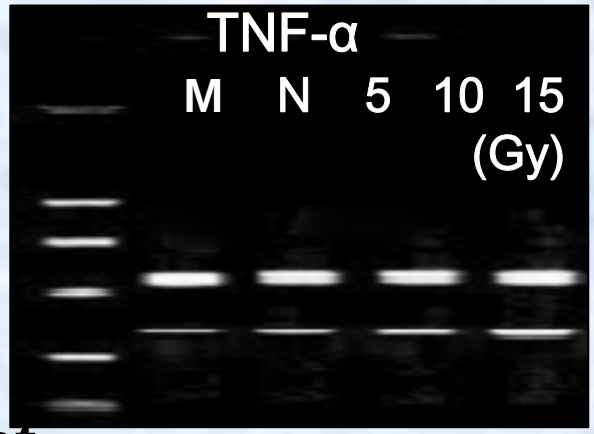


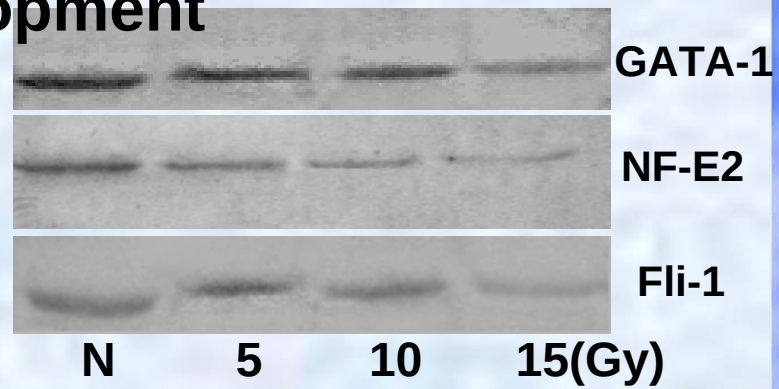
Migration of PMNs towards Meg Transwell assay

Gene expression of Meg after IR

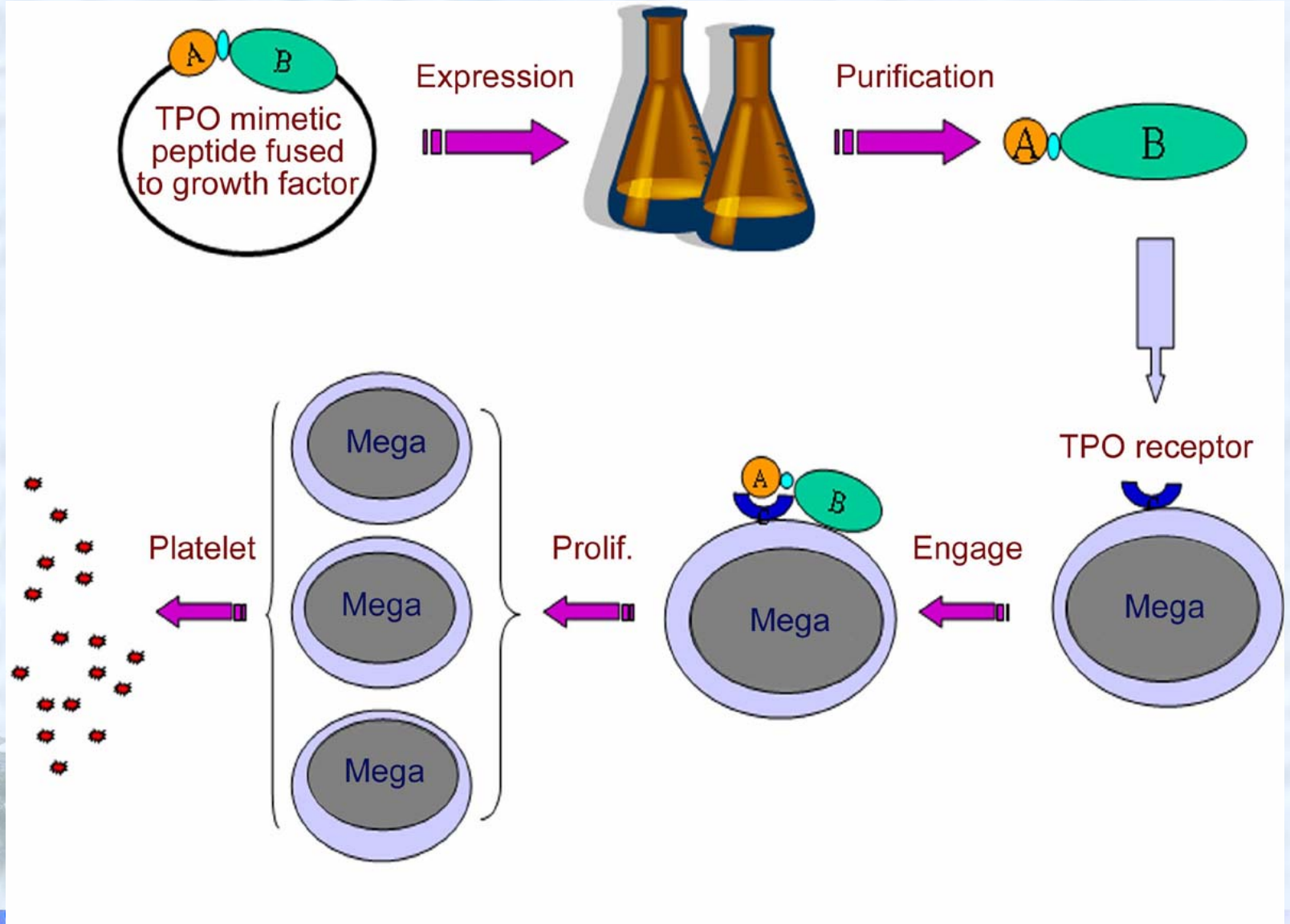
- ↓Genes that *stimulate* the proliferation of meg
  - IL-3, IL-3R, GM-CSF, IL-6
- ↑Genes that *inhibit* the proliferation of meg
  - TNFα, TNFαR, TGFβ1
- ↓ TFs that regulate meg development (mRNA & protein)
  - GATA-1, NF-E2, Fli-1



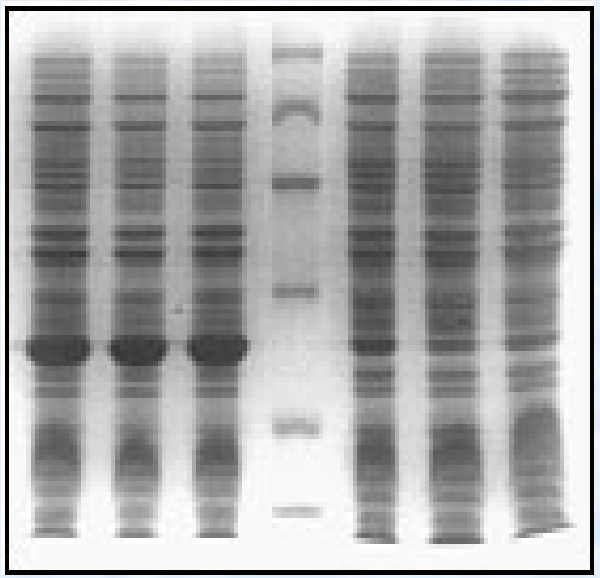


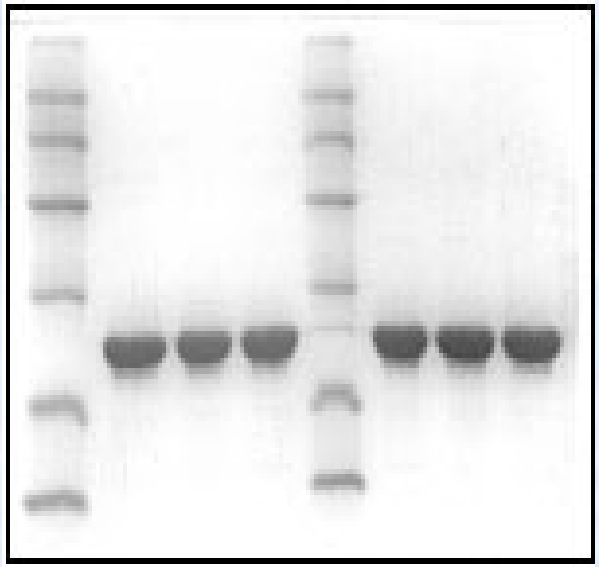


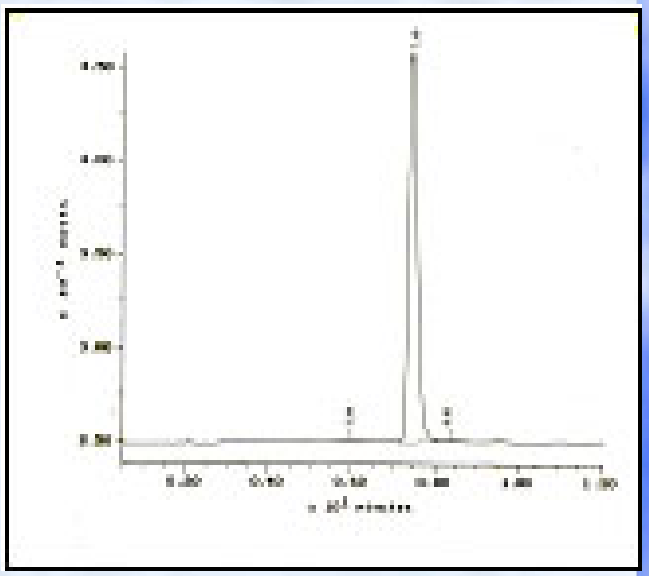
New type of fusion protein stimulating thrombogenesis(FPST)



Expression Induction and purification



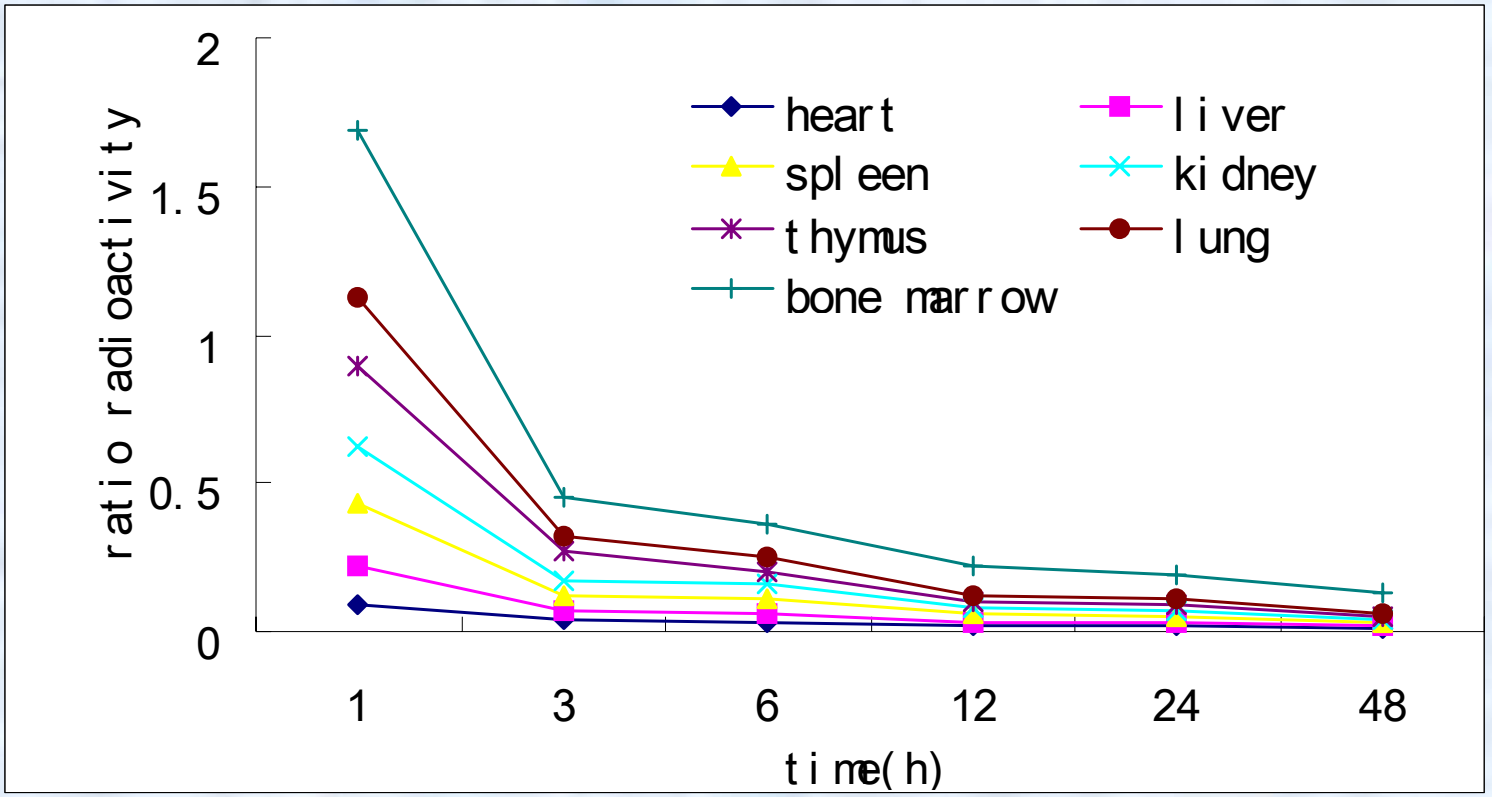




Induction **Purification** **HPLC**

The MW was as expected and a.a. sequence confirmed by N-terminal sequencing

The distribution of FPST after i.v.



The highest conc. of 125 I labeled FPST is in blood within 3h after i.v. and there after in BM, where it take effect.

Test the immunogenicity of FPST

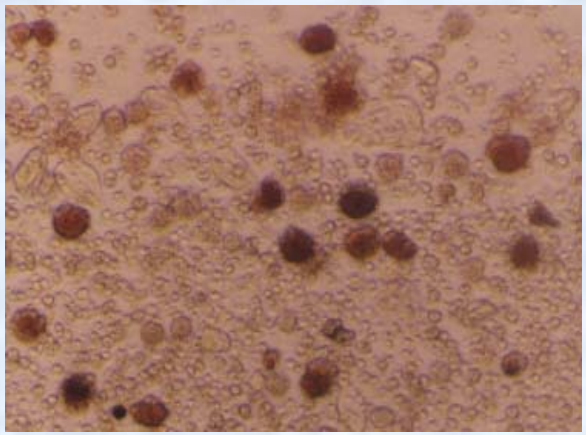
groups	ELISA	gel diffusion
OVA+adjuvent ×4	1.1512**	+
FPST+adjuvent×4	0.009	-
FPST 40ug/d×15d	0.007	-

No obvious immunogenicity detectable

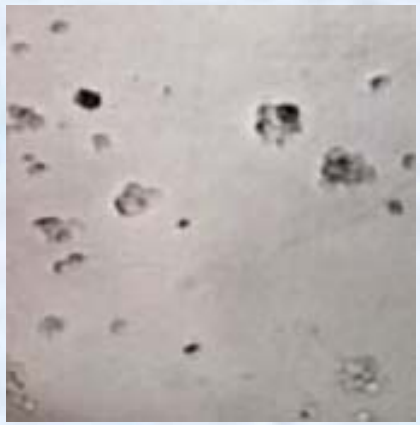
Bioactivity of FPST tested in vitro

Significantly increase the production of CFU-Meg

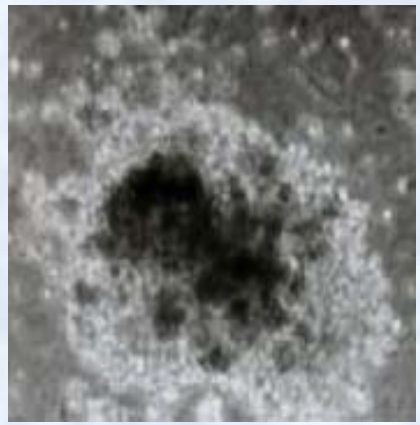
Supportive effect on CFU-E and CFU-GM



CFU-Meg

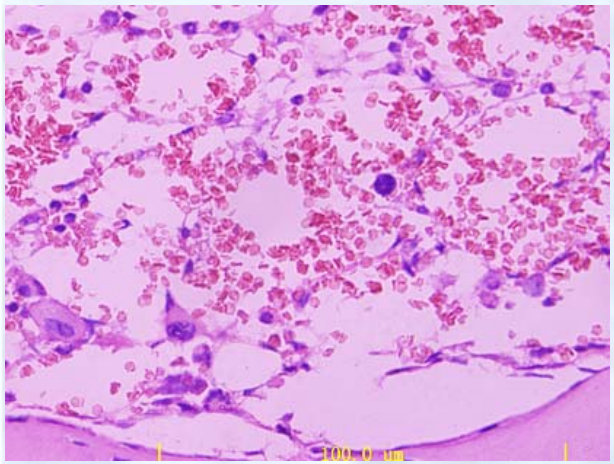


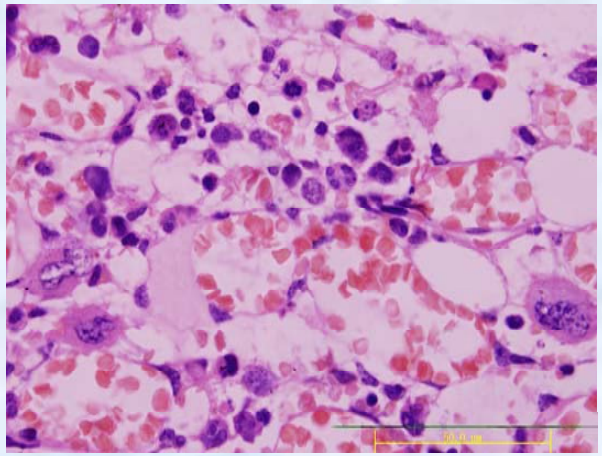
CFU-E

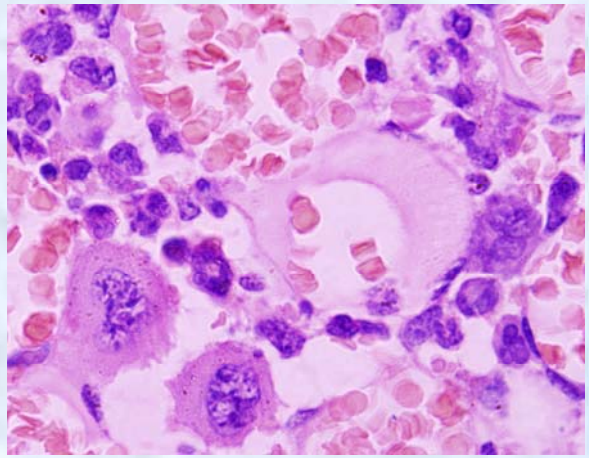


CFU-GM

Bioactivity of FPST tested in vivo (10Gy+15%TBSAIII°)



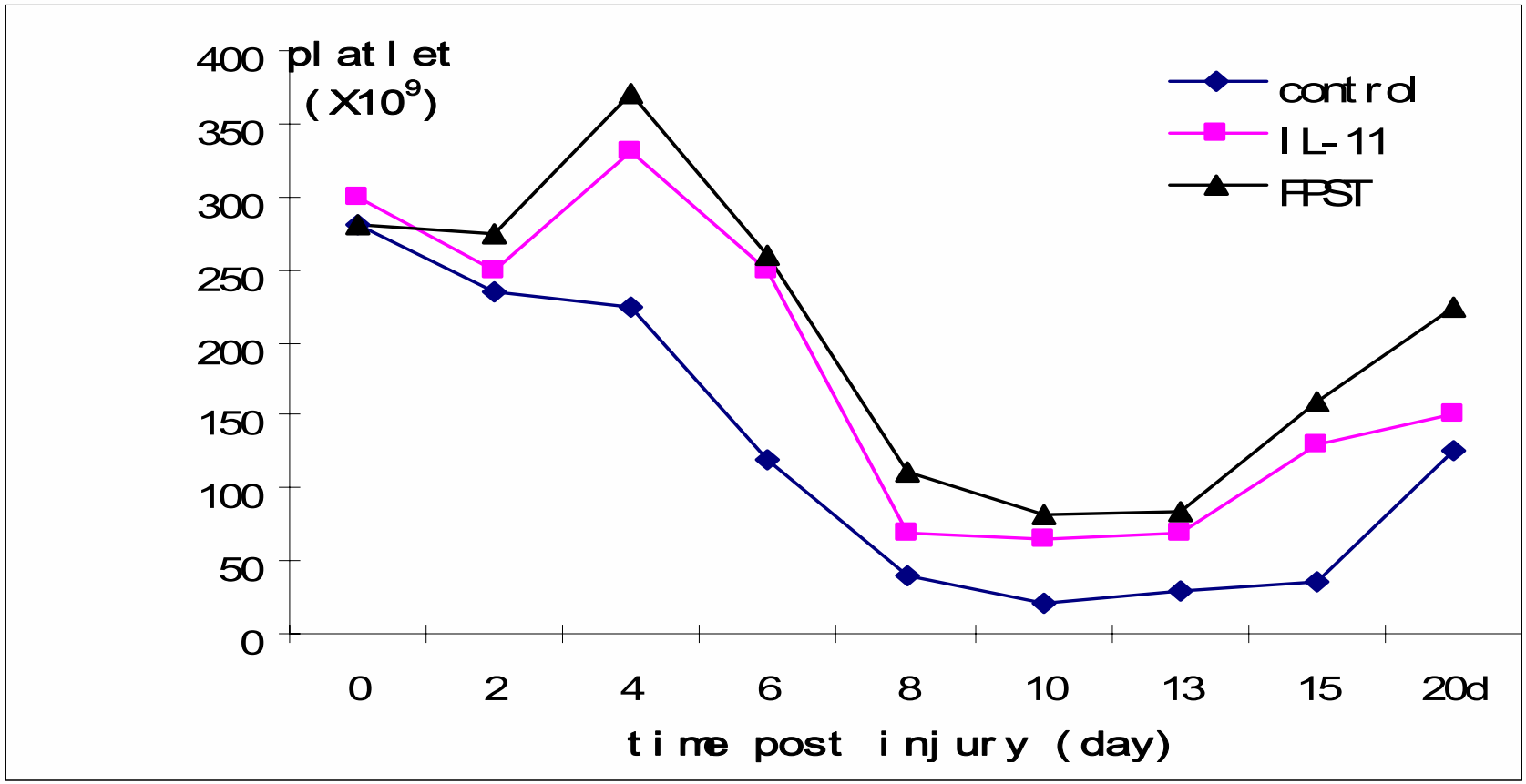




Untreated **Treated with FPST**

Promote the hematopoiesis recovery accompanied by more newly produced Meg from extremely severe radiation-burn combined injury

Peripheral platelet increased



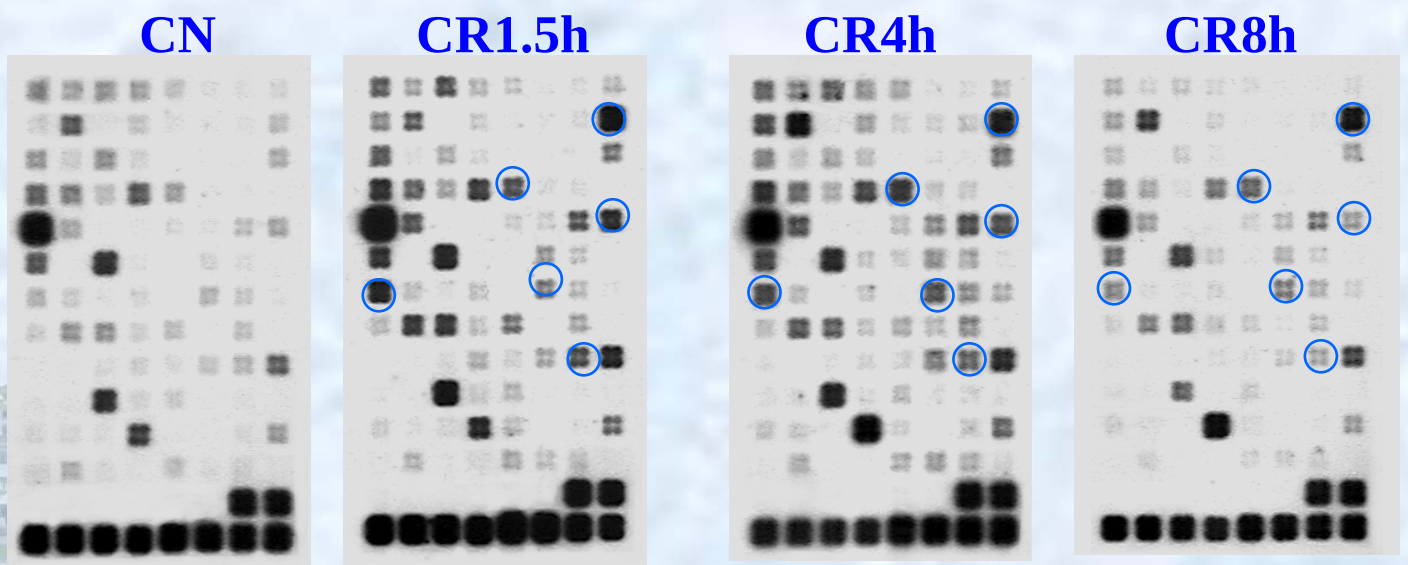
better than IL-11

2. Damage and repair of intestinal epithelium

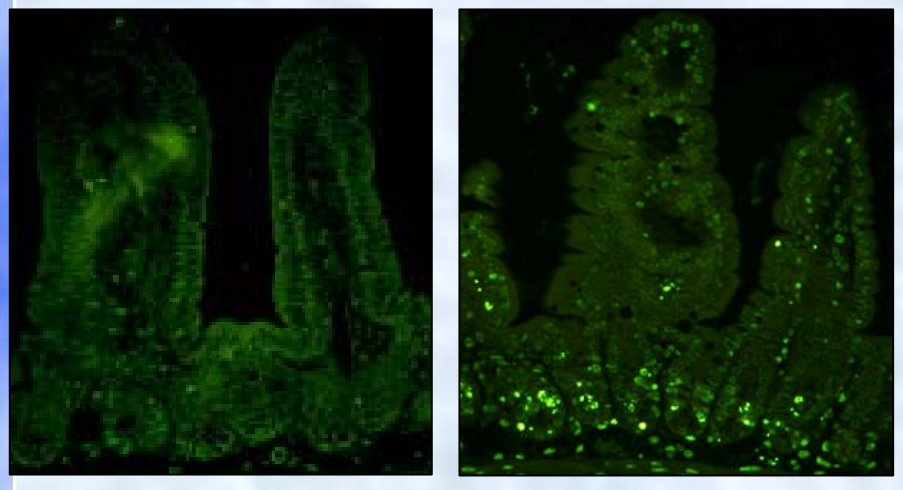
- The molecules determining the ***combined effect*** in intestinal epithelium were clarified
- New measurements to prevent and treat gastrointestinal tract injury were proposed.

GEArray Q Series Mouse Gene Array

- p53 pathway showed changes in expression after IR
  - mdm2, puma, Cyclin D, Gadd45 tuned up
- NF-κB pathway showed changes in expression after IR
  - NF-κB2, Rel A, I-κB , A20 tuned up



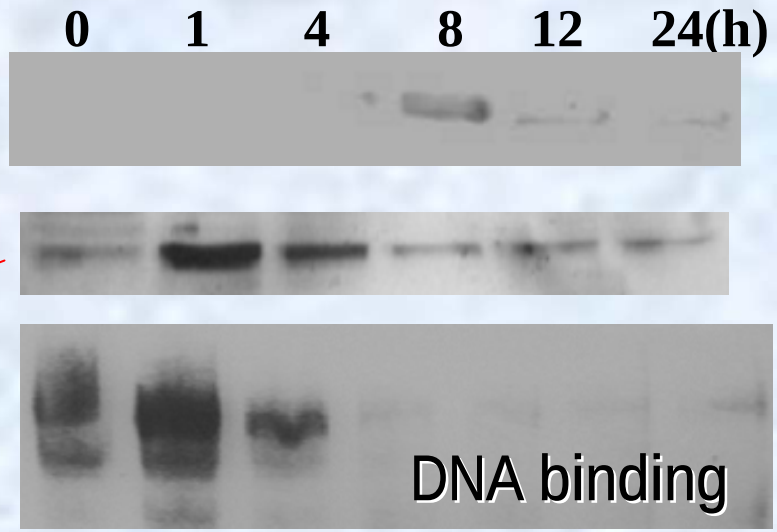
p53 play an important role in the damage of intestine epithelium



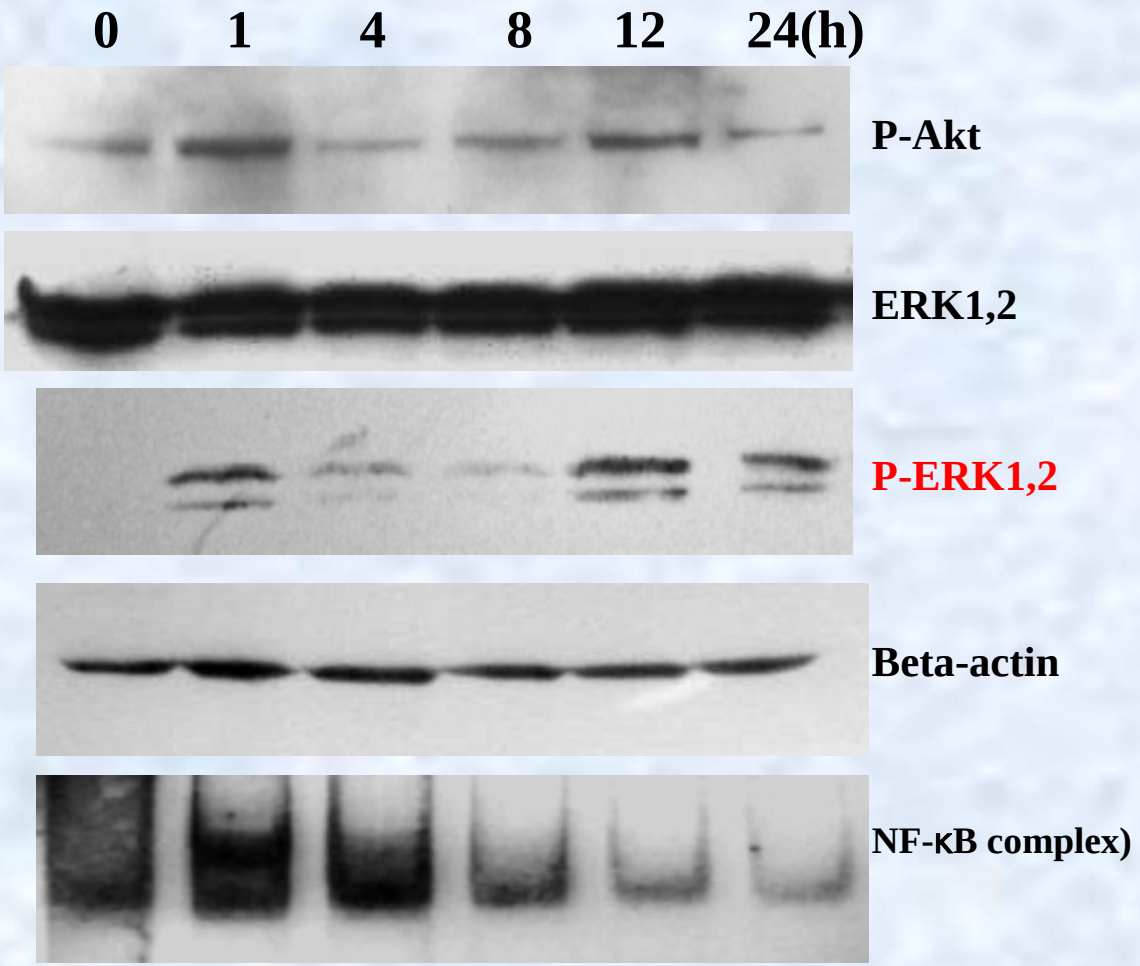
Normal 12Gy IR

TUNEL for apoptosis in intestine

The expression and DNA binding capacity of P53



NF-κB and its related genes changed in crypt after 12Gy IR



The transactivity of NF-κB increased significantly and the upstream p-Akt slightly， ERK1,2 were phospharylated

It was found that the damage to intestinal epithelium (IE) was severer in radiation injury than in RCI and the mechanism of this alleviative effect was deciphered

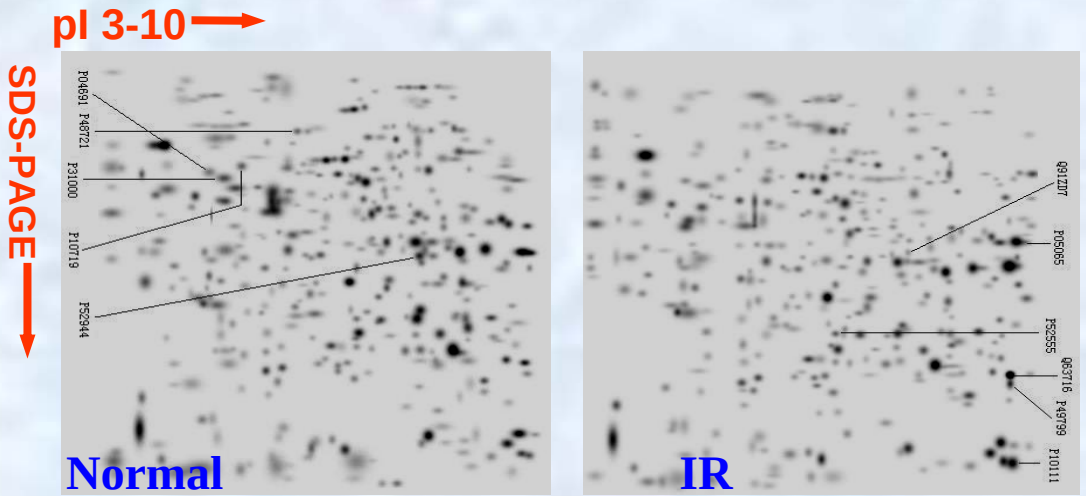
Proteomical study depicted the candidate genes

32 differentially expressed proteins revealed by 2-D gel eletrophoresis were analyzed by MS. After IR the expression of Peroxiredoxin 1, ERp29 (Endoplasmic reticulum protein) increased in both in vitro and in vivo models

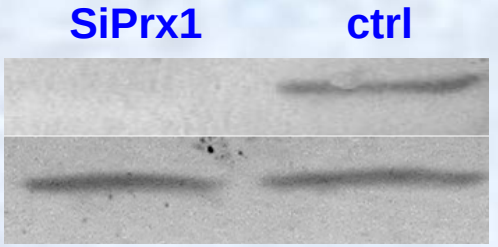
injury：

IEC-6 cells (25Gy)

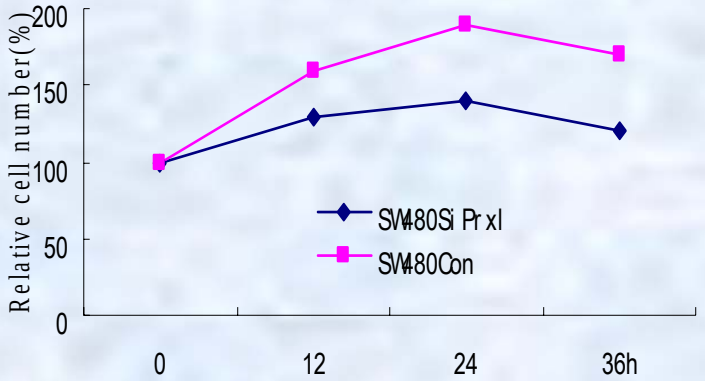
Bal b/c mice(9Gy)



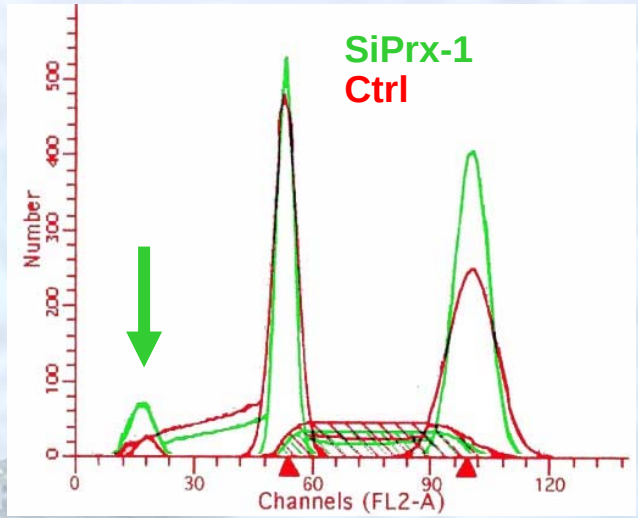
The possible functions of Prx-1



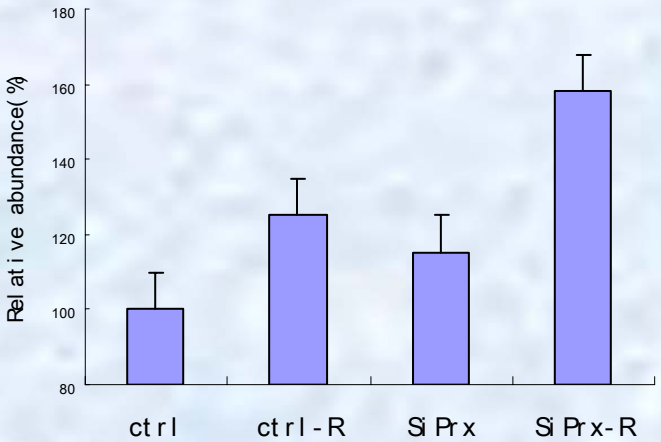
Prx-1 knockdown



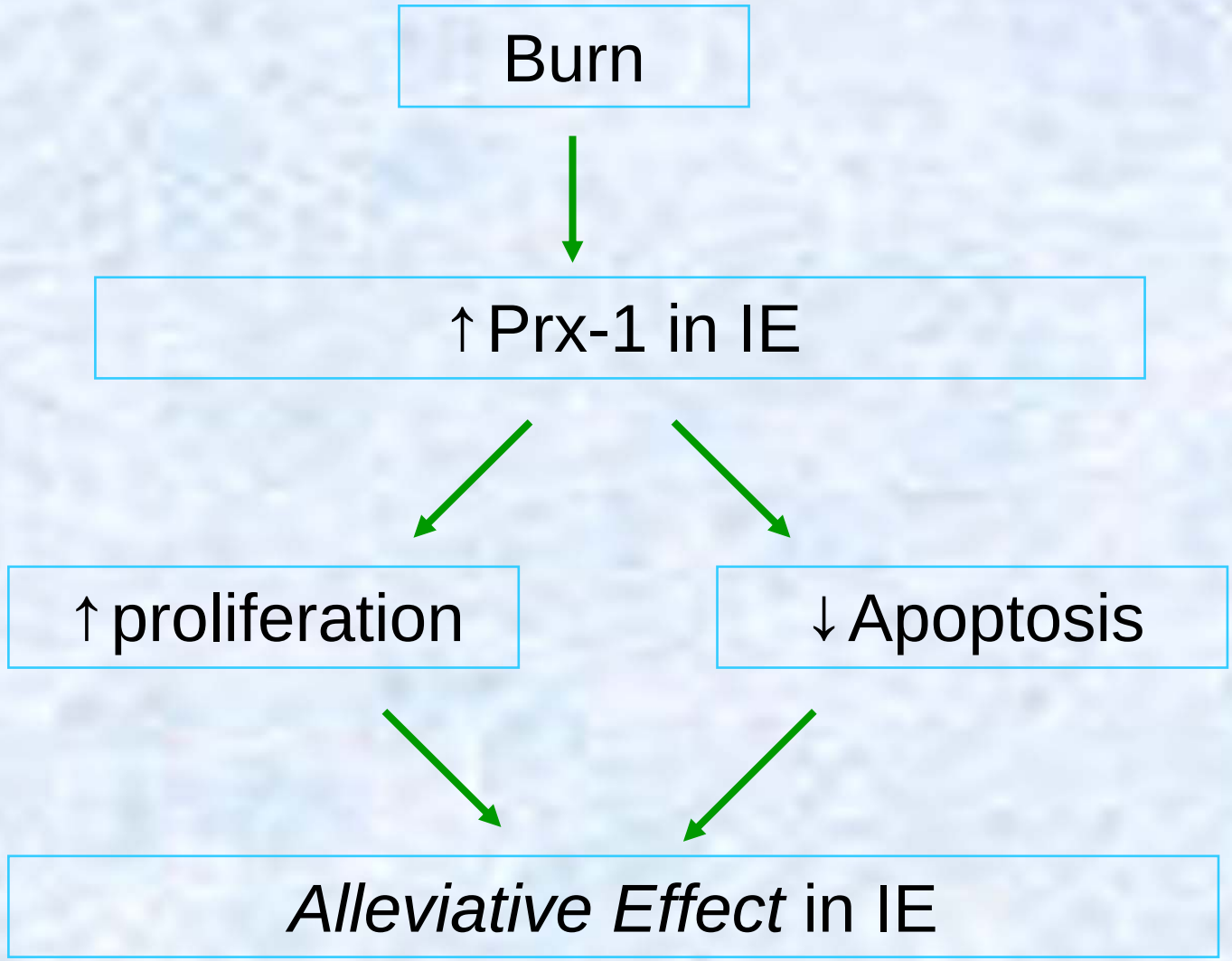
Cell proliferation assay



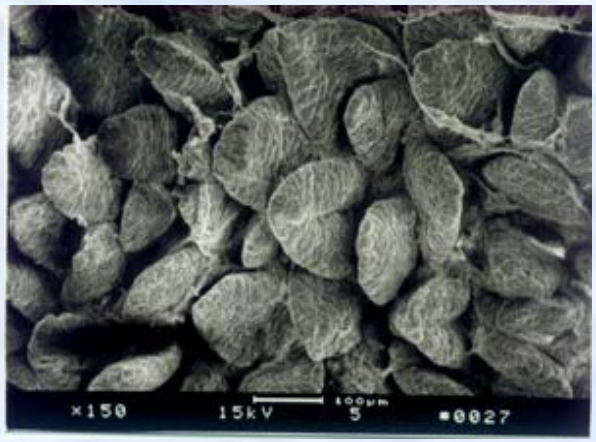
Apoptosis after 10Gy IR



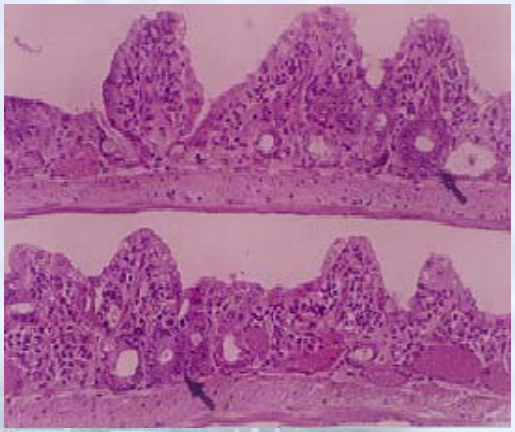
MDA content



Treatment for intestine damage in RCI was proposed



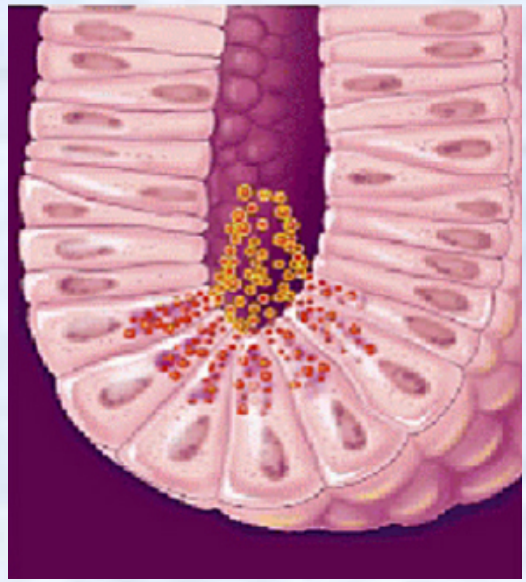
Normal intestinal epithelium



Pathological changes of intestinal epithelium after RCI

Antibacterial peptide, human defensin5—clone and expression

HD-5 broad antimicrobial activity against various bacteria and yeast in vitro

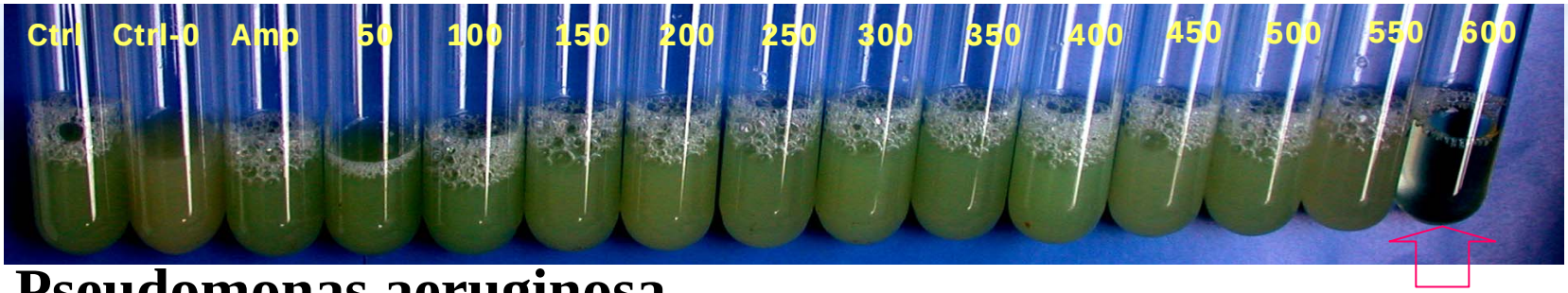


Neutralize endotoxin

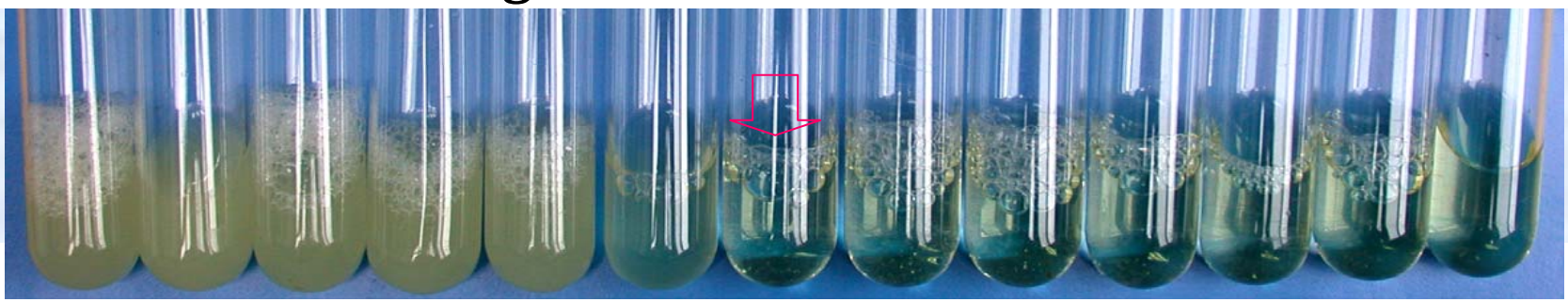
Do not disturb the homeostasis of intestine microbial

Do not digested by intestine enzymes

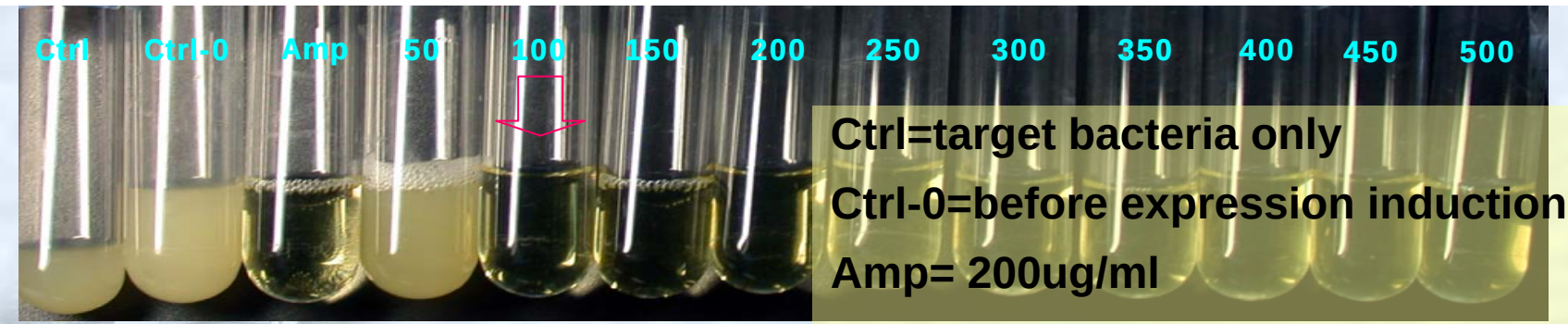
Antibacterial activity of recombinant hD-5 supernatant



Pseudomonas aeruginosa



Acinetobacter baumannii



Staphylococcus aureus

Ctrl=target bacteria only

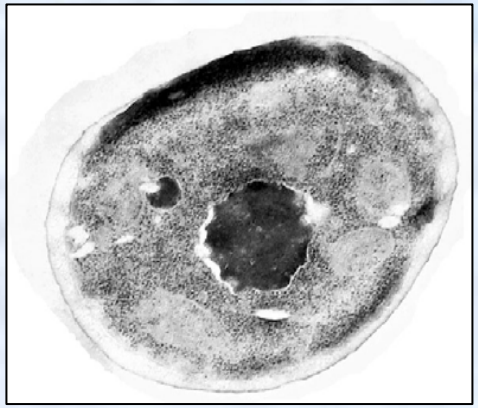
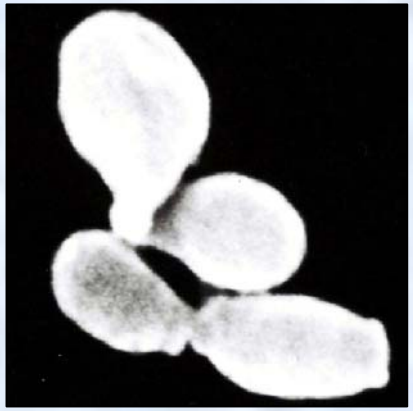
Ctrl-0=before expression induction

Amp= 200ug/ml

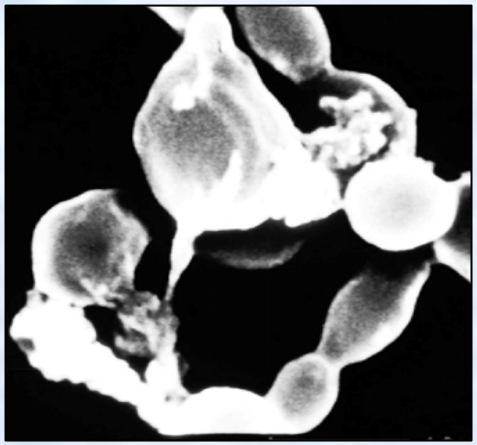
50…=vol. (ul) of supp. after induction

Arrows show the least effective doses

Pathological changes of yeast after rhD-5 treatment

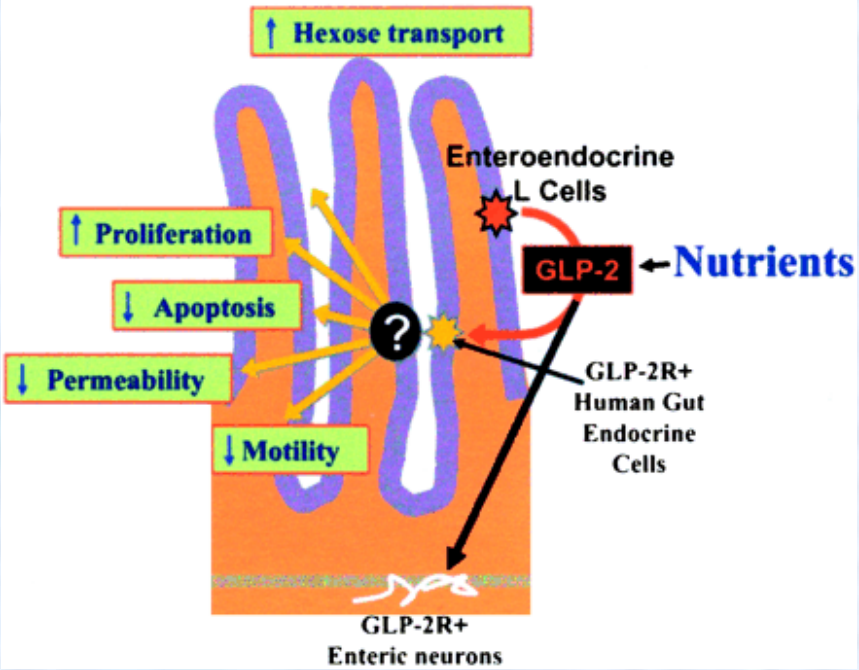

Normal *candida albicans*

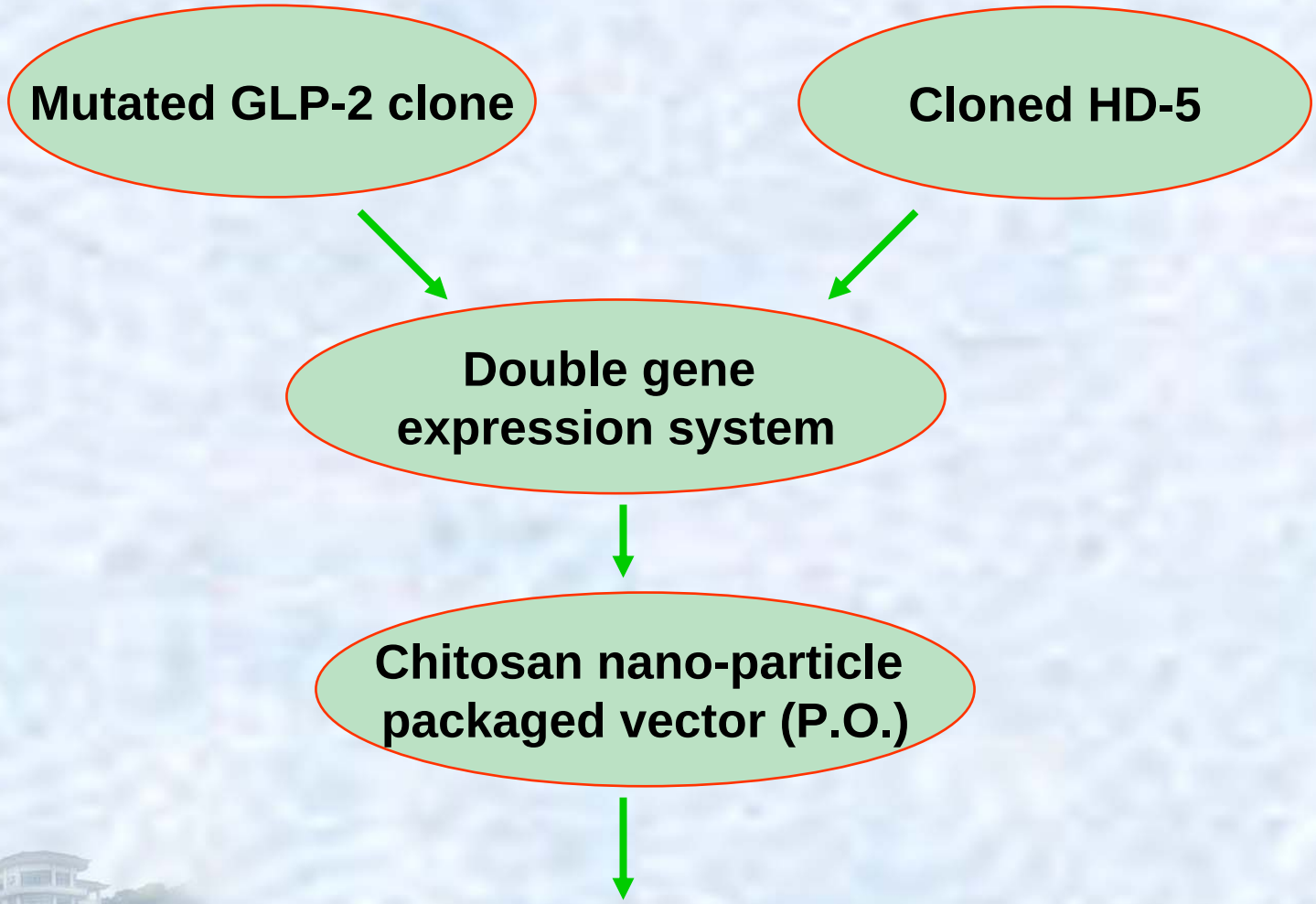

***Candida albicans* 3h after treated by rHD-5**

Glucagon-like peptide-2 (GLP-2)

- ↓Expression after radiation injury(8Gy)
- Exogenous GLP-2
  - ↑ MAPK activation
  - ↑ BrdU incorporation
  - ↑ Cyclin D1 expression
  - ↑I.E. repair (better than EGF)

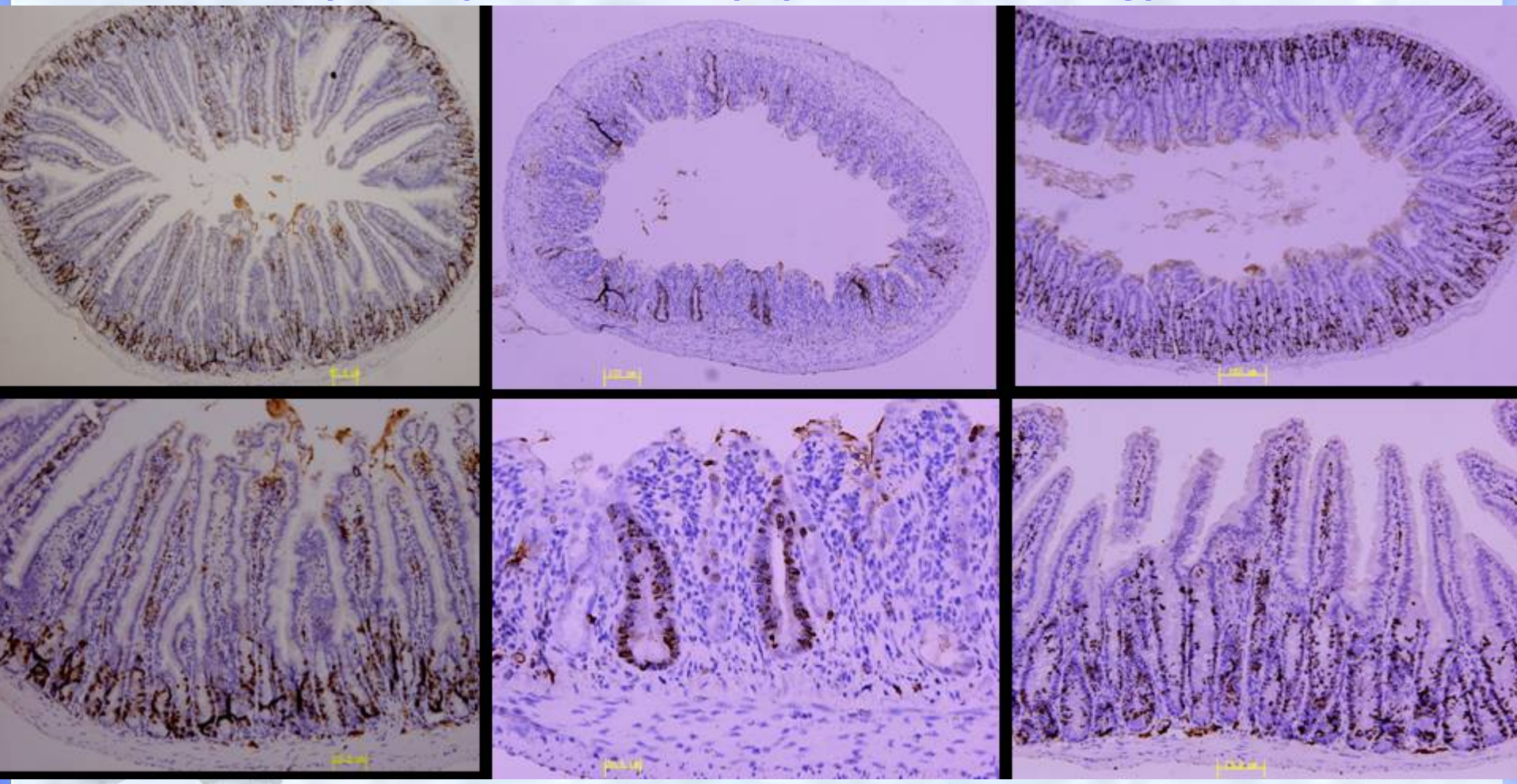


Combining methods in managing GI injury



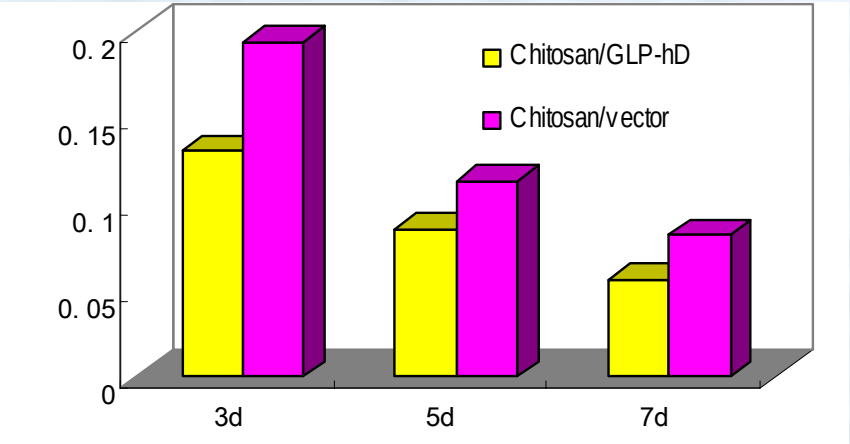
Structure repair—enhancing immunity of intestinal mucosa

Promoted IE repair by GLP-2—HD-5 after RCI (10 Gy+15% III°) (BrdU staining)

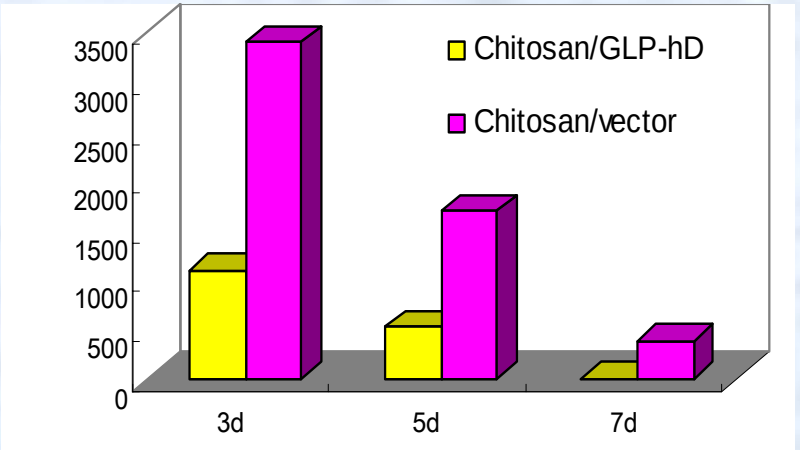


Normal **ctrl vector** **Double genes**

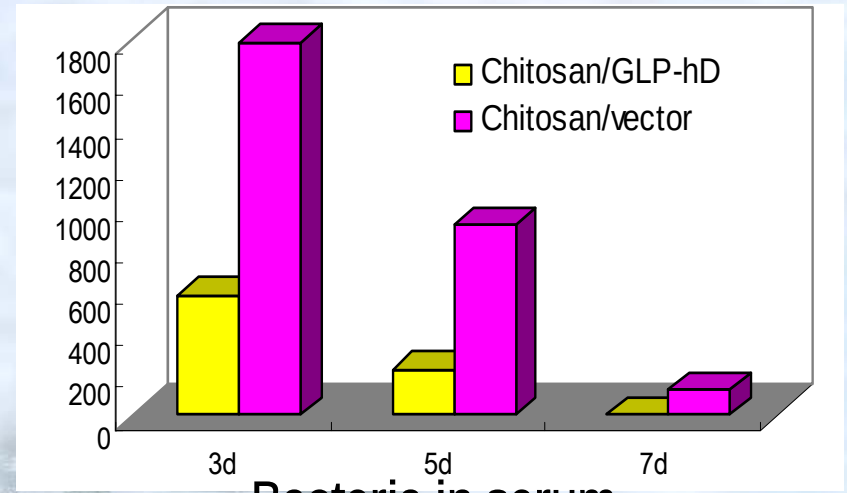
GLP-2—HD-5 co-expression reduced the gut-borne infection



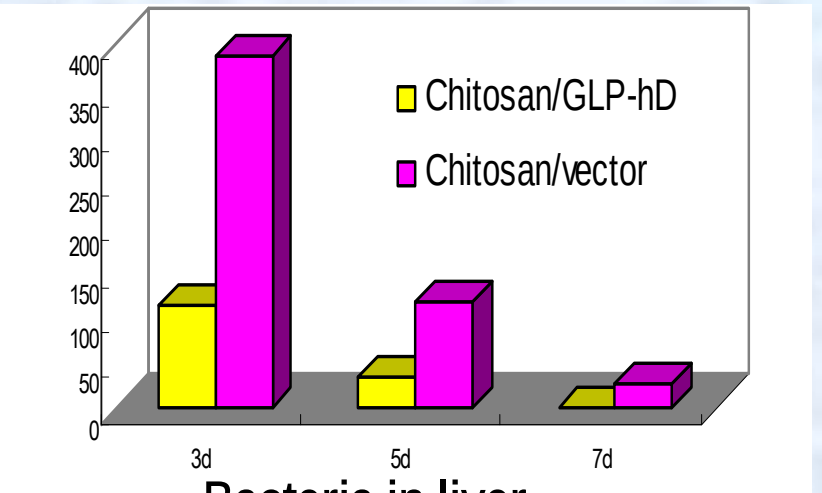
Endotoxin in serum



Bacteria in mesentery lymph node



Bacteria in serum



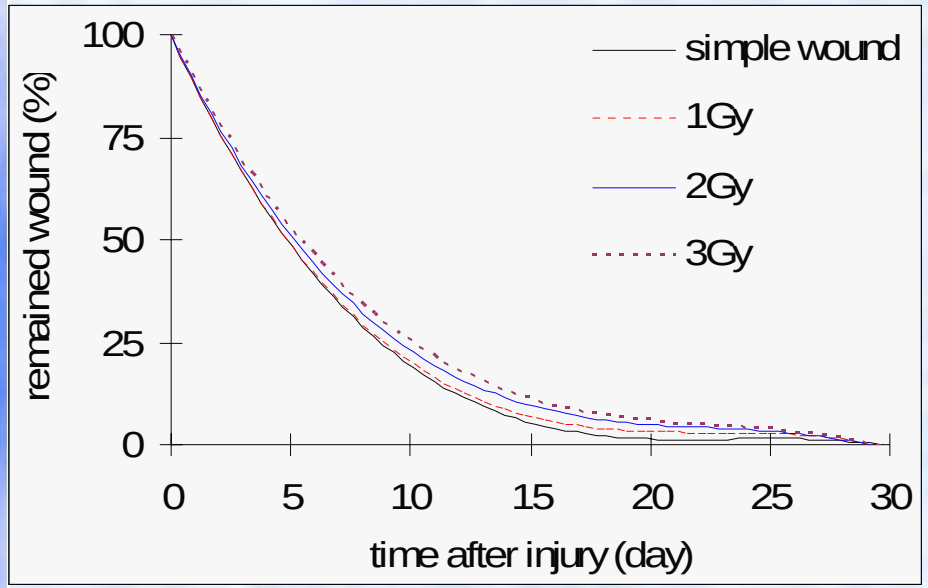
Bacteria in liver

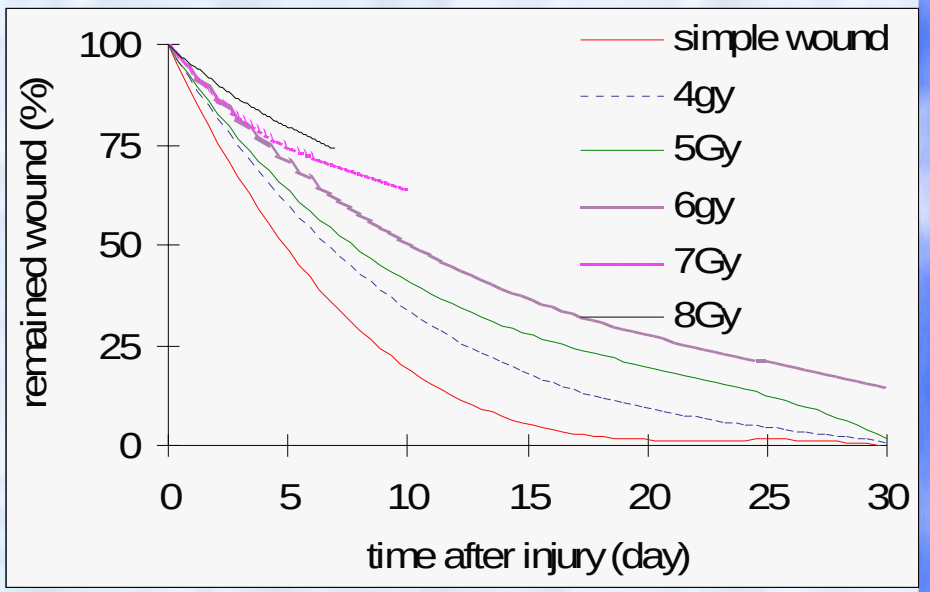
3. On difficult wound healing and promoting wound closure

some of the main causes for refractory wound of RCI were unveiled and some effective methods to accelerate wound closure were tested

Relationship of wound healing delay with time point and IR dose

(1-8Gy, 3.8cm^2 full thickness skin removal)





Wound healing delay not obvious when combined with 1～3Gy，proportional to IR dose when with 4～6Gy. Animals died soon after 7、8Gy IR

Formula to describe relationship of wound healing delay with time point and IR dose of TBI

$$\hat{Y}=64.139-3.703d-2.935t$$

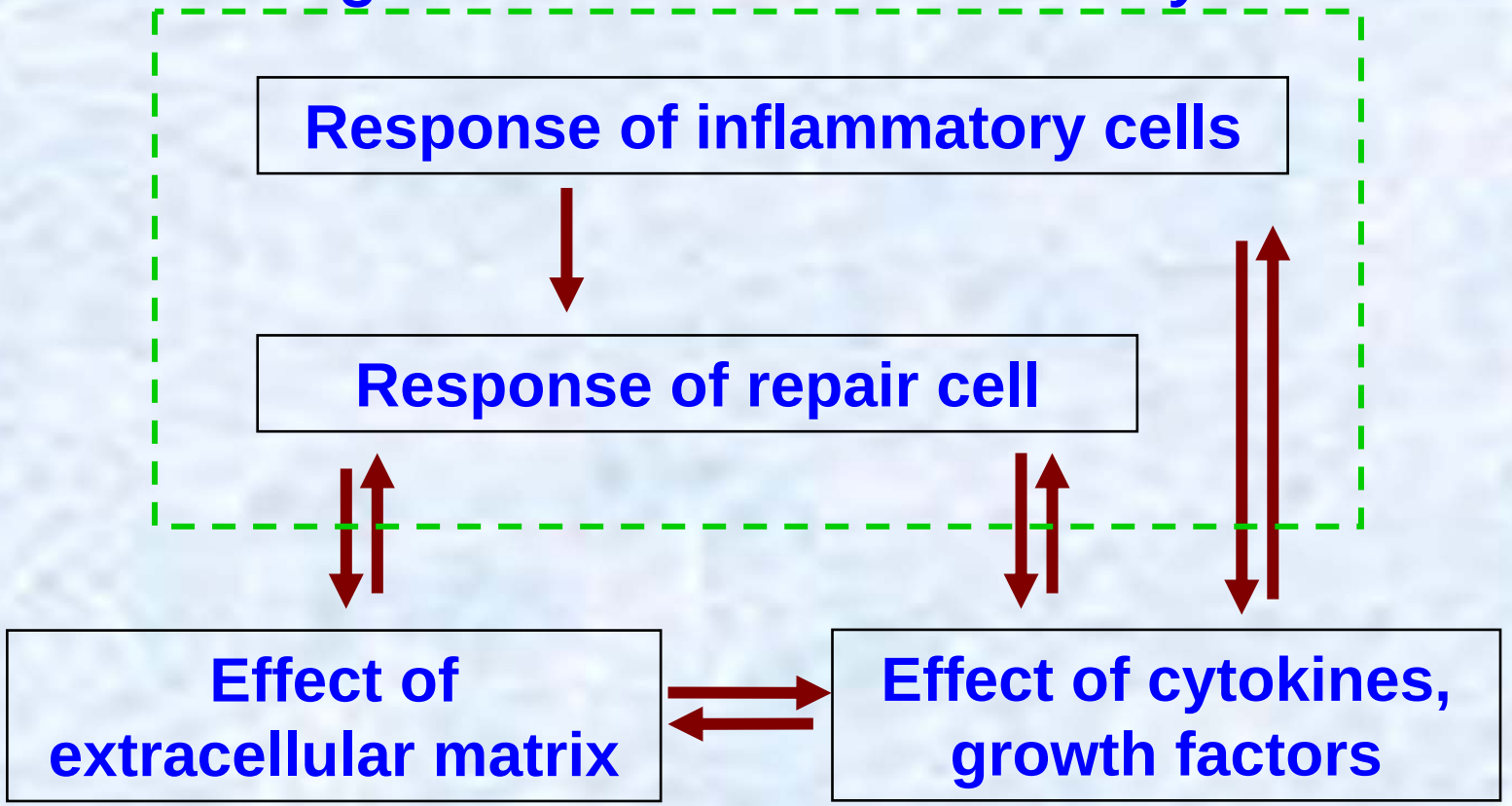
$\hat{Y}$ remained wound (%)

d absorption dose (Gy)

t time after injury (day)

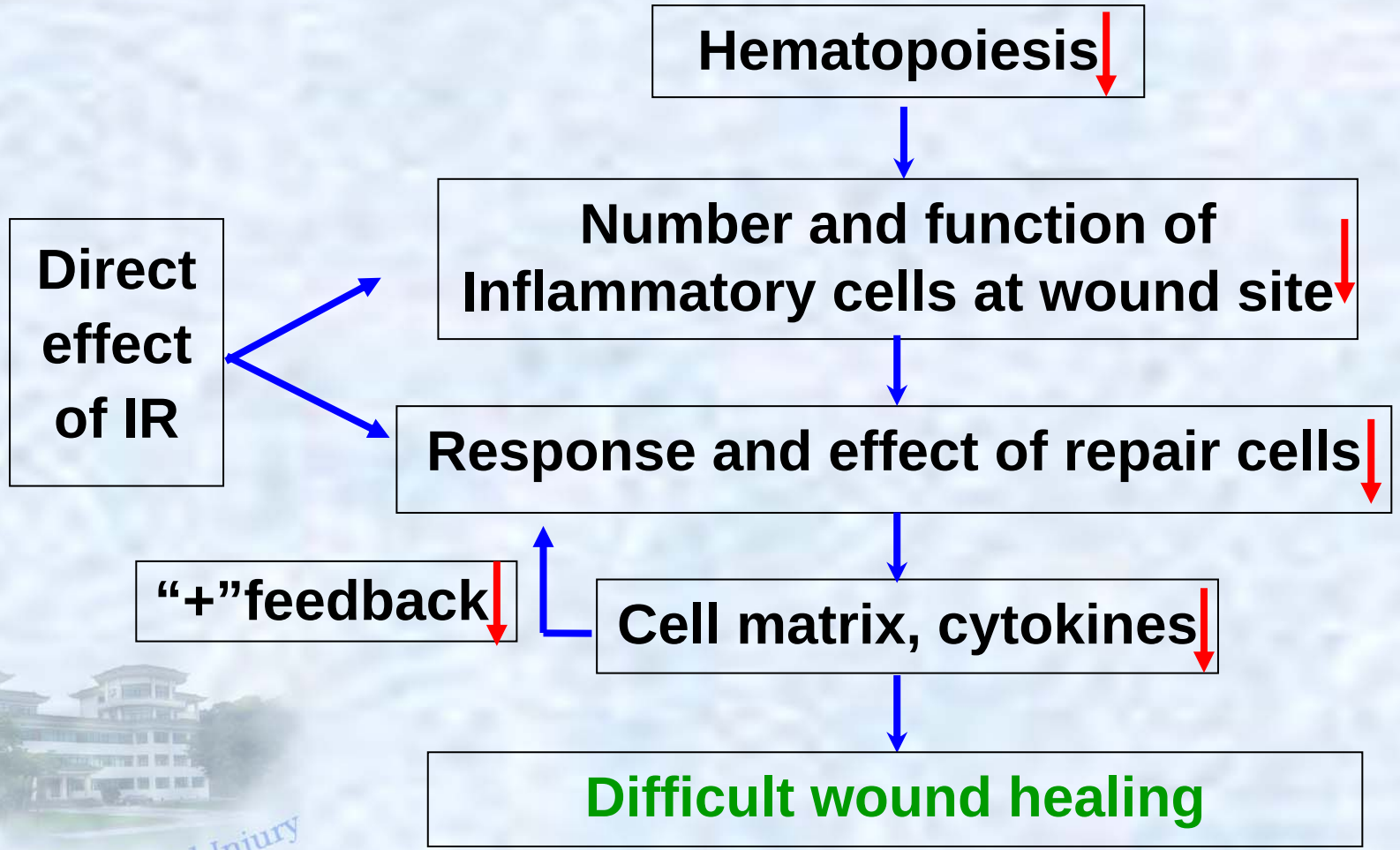
Useful for estimation on wound healing delay of large population

The changes and their causing factors related with wound healing were studied individually and collectively



The mechanism of radiation-wound combined injury was proposed as:

network composed by wound healing-related factors was deregulated with cell damage as a key problem

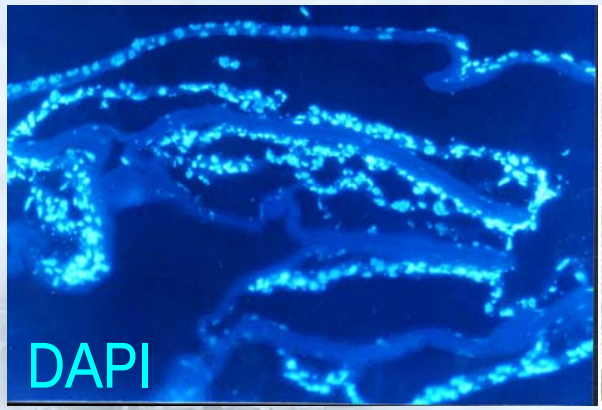


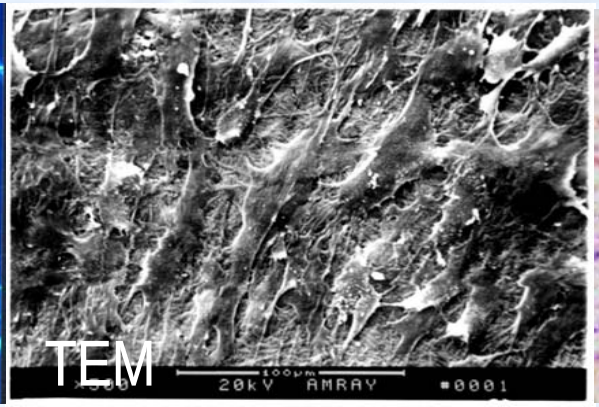
Based on the proposal and referred to clinic experience, regimen for RCI related difficult wound healing was tested

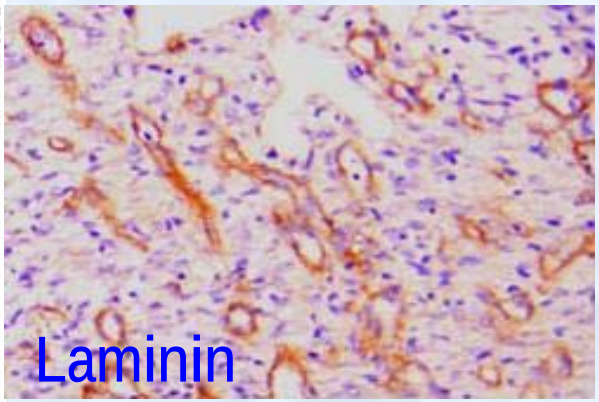
- Phenytoin sodium
- W11-a12 (Chinese herb extracts)
- Neural growth factors
- Compressive external fixation
- Stem cell engineering

Applied stem cells to RCI

- Engineered skin sleeve: Amnion matrix loaded with PDGF-A modified MSC
- Topical use of hBD2 modified dermal stem cells (DSC)
- Transplantation (I.V.) of DSC showed multifunction
- CXCR4 modified MSC and DSC preferentially distribute to wound site







Amnion matrix loaded with PDGF-A modified MSC promote angiogenesis

Bone fracture fixed with pressure healed better

- Improve blood circulation locally
- Accelerate the BMP, bone formation and reconstruction

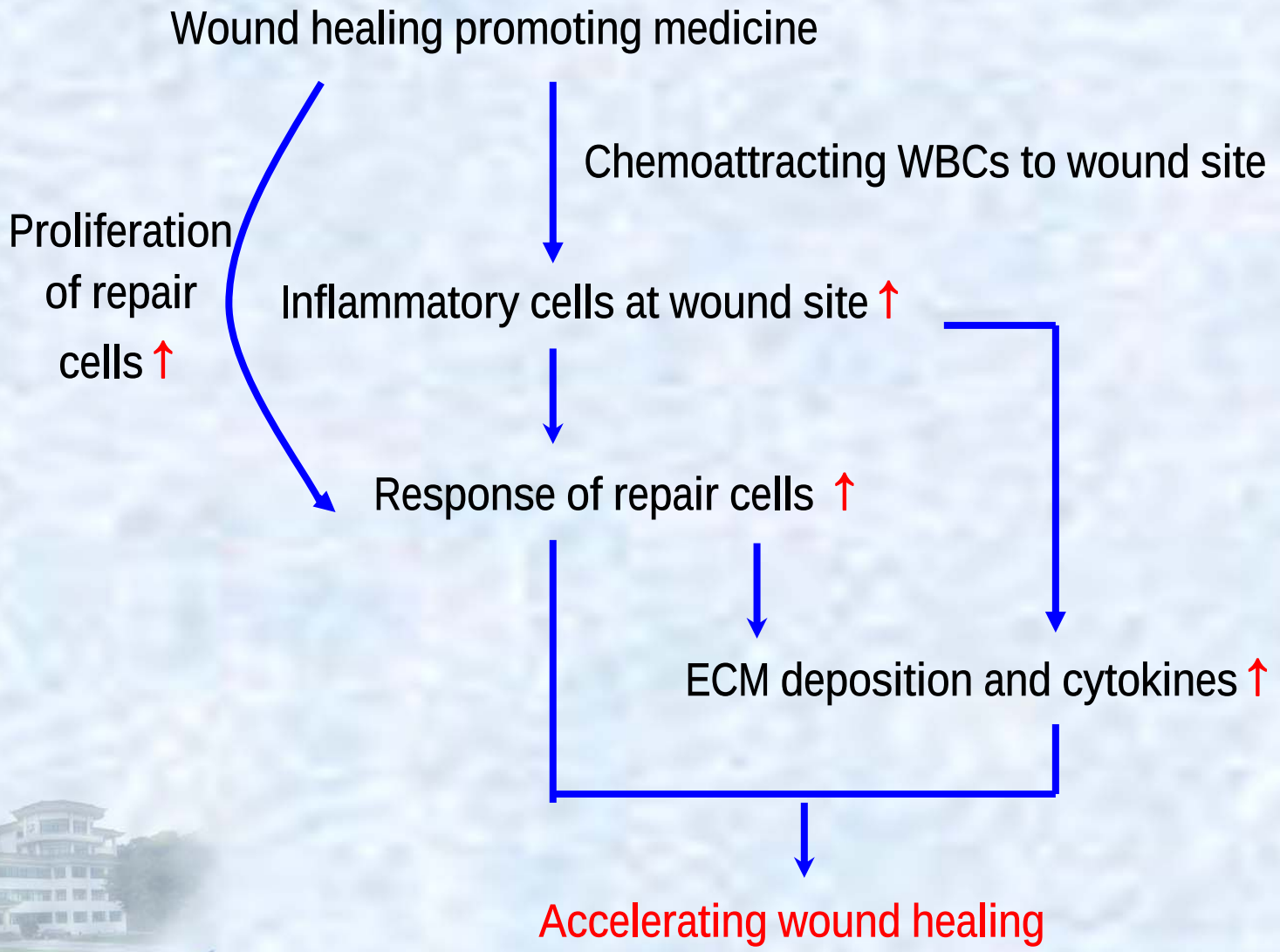
Mini half-ring external compressive fixator
(fracture+20Gy local IR)

Compressive fixator, 12w

Regular fixator, 12 w

Twist force, 2X

How was the difficult wound healing of soft tissue improved



eschar excision and skin grafting

Based on the idea that IR inhibit the reject reaction of immune system, the determined appropriate time for eschar excision and skin grafting was of significance for clinical treatment of the injury

Skin graft after RCI

Survival of grafted allogenic skin at day 30

Abs. dose (Gy)	0	4	5	6	8*
Survived graft	0	10%	36%	42%	100%

*allogenic BMT at the same time

Principle of skin grafting (1)

- Large-size partial thickness autogenic grafting with in 24h survived and mice survival rate reach 95%
- Grafted allogenic skin could not survive if grafted during 24-72h after injury
- Grafted allogenic skin rarely survived if perform eschar removal and skin graft during critical phase

Principle of skin grafting (2)

- Transfusion of stored or irradiated blood may inhibit immune response, which may help grafted skin survival and hematopoietic cell engraftment
- Blood circulation of grafted skin initiated at 3-4 day and joined with acceptor' skin around 10 days
- Skin grafting was practicable during recovery phase

Our research focused on the key problems in the development and regression of RCI, theoretically the mechanism of combined effect was clarified and practically some novel treatment were tested

The above advancements of our research are obviously meaningful for on site emergency rescue and in hospital treatment of RCI

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